SYNOPSIS

"Cardiovascular and hepatic implications of Monosodium glutamate (food flavour enhancer) exposure: Ameliorative efficacy of α-tocopherol"

Changes in food consumption patterns and advances in food processing technology have led to the use of flavours in food. Flavours have the ability to improve the taste of a wide variety of foods that are not very tasty. Flavourings are often used in junk foods to mask the absence of natural ingredients and increase the taste of foods. Approximately 3000 different chemicals are added to foods during their manufacturing to improve taste, flavour etc. Food additives are chemical substances added intentionally to food stuffs to preserve, colour, sweeten and flavour food. Most of the components used in food additives are associated with varying levels of negative health implications. Monosodium glutamate (MSG) is the sodium salt of naturally occurring non-essential L-form of glutamic acid and it contains 78% glutamic acid, 22% sodium and water (Adrienne, 1999). MSG was discovered by a Japanese scientist, Kikunae Ikeda in 1907. He extracted and identified the flavour enhancing property of glutamate from traditionally used seaweed *Laminaria Japonica* and patented in 1909 by Ajinomoto Corporation in Japan. It does not have a distinct taste of its own, but provides a flavouring function when it is added to food. This characteristic taste is called "umami", which is considered distinct from the four basic tastes (sweet, sour, salty and bitter) (Yamaguchi and Ninomiya, 1998). When MSG is added to foods, the palatability of food is increased by stimulating the sense of taste which are mediated through glutamate receptors located on the taste buds (Nelson et al., 2002). MSG is incorporated in large number of solid and liquid foods especially in soups, sauces, snacks, processed, packed and restaurant foods and it is known by the trade names of Ajinomoto, Sasa, Ve-tsin, Miwon, Weichaun and tasting powder (Fuke and Shimizu, 1993).

The first published report of an adverse reaction of MSG was described by Dr. Robert Ho Man Kwok in 1968. He coined the term Chinese Restaurant Syndrome (CRS) which began 15-20min after eating the first dish at a Chinese restaurant and lasted for about 2hrs. According to Kwok, the most prominent symptoms were numbness at the back of the neck gradually radiating to arms and back, headache, tingling, flushing, muscle tightness and weakness

(Kwok, 1968). In addition to these complications, ingestion of MSG has been alleged to cause asthma, atopic dermatitis, ventricular arrhythmia, neuropathy and abdominal discomfort (Geha et al., 2000). Glutamate has been a trigger for migraine and headache exacerbations (Woods et al., 1996). Radnitz (1990) described that glutamate initiated a vasomotor reaction, which caused throbbing pain across the forehead and suggested that those who experience migraine and headache were more susceptible to headache triggered by glutamate. The FDA received no reports of unconsciousness, coma or death related to ingestion of MSG but 8.2% of the evaluated subjects were severe and experienced difficulty in breathing, chest pain, changes in heart rate and blood pressure. In 1995, Federation of American Societies for Experimental Biology (FASEB) admitted the possibility of MSG as a causing agent of food intolerance. Life threatening reactions associated with consumption of MSG include anaphylaxis, seizures, dysrhythmias, constricted throat, dyspnoea, head or neck edema, hypovolemic shock and syncope (Raiten et al., 1995).

Acceptable daily intake (ADI) of MSG is 0-120 mg/kg body weight and it is not applicable to infants under 12 weeks of age because blood brain barrier is not fully developed in infants and cannot protect against toxins that enter the blood (FAO/WHO, 1974). The good taste of MSG added foods caused people to consume higher dosage of this compound (Isa and Ghani, 2009). The MSG consumption has increased globally in recent years because of increased uptake of processed food products. It is frequently added to processed foods and mixed onto foods during preparation. The average daily glutamate intake from food additives may reach up to 1g in Europe, 4g in Asian countries and 10g in Germany (Beyreuther et al., 2007). In Nigeria, most communities and individuals use MSG as a bleaching agent for the removal of stains from cloths. Its excellent bleaching properties could be injurious to tissues and organs of the body (Inuwa et al., 2011). There is lack of evidences for determining whether or not long term oral consumption of MSG caused tissue level damages. Most of the animal studies were conducted through parenteral route within a very short period of time and very fewer studies have assessed the cardiovascular and hepatic complications. Thus the first aim of current investigation was to examine the effect of chronic oral consumption of low and high doses of MSG on cardiac and hepatic tissues.

The experimental rats orally received MSG at different low (50 and 100 mg/kg b.wt) and high (4 and 8 g/kg b.wt) doses for 180 days. Concentration of plasma aminoacids, cardiac function parameters (aspartate aminotransferase, lactate dehydrogenase and creatine phosphokinase), serum electrolytes, liver function markers (alanine aminotransferase, alkaline phosphatise, γ-glutamyl transferase, protein and albumin), lipid peroxidation markers in plasma and tissues (malondialdehyde and conjugated diene), antioxidants level in hemolyzate and tissues - superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx) and glutathione transferase (GST); histology and genotoxicity in heart and liver were monitored.

The results revealed that the deleterious effects of MSG were dose dependent and plasma glutamate levels were increasing as dose increases. The experimental rats received low doses of MSG maintained normal levels of serum electrolytes, functional markers and normal architecture of cardiac and hepatic tissue. Increase in the lipid peroxidation at 100 mg/kg dose of MSG exposure in the present study was counteracted by corresponding increase in the antioxidants mainly SOD and CAT in heart and liver. This suggests that the lower concentrations of MSG were not enough to induce hepatotoxicity and cardiotoxicity. Rats were treated with high doses of MSG showing significant increase in plasma glutamate, liver function markers, cardiac function markers, serum calcium level and lipid peroxidation with significant decrease in antioxidant status as compared with control. Lipid peroxidation and oxidative stress after high doses of MSG treatment may be due to the glutamate toxicity, which is associated with over-excitation of glutamate receptors. Excessive accumulation of glutamate in extracellular spaces and subsequent activation of glutamate receptors play a key role in the pathophysiology of oxidative stress related neuronal injury (Monnerie et al., 2003). Glutamate receptors were once thought to be predominantly located only in the central nervous system (CNS), but further studies revealed their presence in a variety of peripheral neural and non-neural tissues including heart and liver (Gill et al., 2007; Julio-Pieper et al., 2011).

Increased lipid peroxidation and diminished antioxidant potential leads to oxidative damages of cell components (Packer and Landvik, 1990). Light microscopic examination indicated pronounced histological alterations in heart and liver. Swelling, fiber separation and

congestion were observed in cardiac tissue. Vascular congestion and haemorrhages were observed in hepatic tissue. Thus the present study revealed that oxidative stress and free radicals formed in the presence of MSG could be responsible for the tissue lesions in heart and liver. Therefore, chronic and high doses of MSG consumption in rats implicate the presence of oxidative tissue damage.

The increased lipid peroxidation, decreased antioxidant status and associated hepatic and cardiotoxicity in the present study can be related to the insufficient antioxidant potential. In recent years, antioxidant treatment has attained a lot of importance because of their potential as prophylactic and therapeutic agents in many diseases. The intake of antioxidants enhance biological mechanisms and prevent oxidative stress related disorders and organ toxicity (Havsteen, 2002). Currently, there is a rising interest in the natural antioxidants due to their low toxicity. Thus the use of natural antioxidants and dietary supplements has been the object of many studies. Antioxidant vitamins play an important role in the regulation of physiological and pathological processes by enhancing the immune system, modifying carcinogen metabolism, altering cell proliferation, stimulating the repair of carcinogen induced DNA damage and elicit free radical scavenging properties (Chaudiere and Ferrar-Iiou, 1999). Vitamin E is a major lipid soluble nutritional antioxidant and can trap organic free radicals and protect proteins and lipids in biological membranes from oxidative damage (Yatin et al., 2000). α-tocopherol (ATC) is the most abundant and highest biologically active form of vitamin E, which inactivates harmful free radicals (McDermott et al., 2000). Major dietary sources of α-tocopherol include animal fats, vegetables, meats, fruits, nuts, cereals and dairy products. In view of antioxidant and cytoprotective potential of ATC, it needs to be explored against MSG induced oxidative damage. Thus the second aim of the present investigation was to find out a moderate dose of ATC and to analyze protective potential of ATC against the oxidative stress mediated MSG intoxication.

In order to study the ameliorative effect of ATC, rats received 4 g/kg of MSG and two doses of ATC (100 and 200 mg/kg) by oral gavages daily for 180 days. The doses of ATC used in this study were not toxic as evidenced by normal levels of biochemical parameters and histology. In the present study, rats were subjected to chronic oral exposure of MSG along with ATC at dose of 200 mg/kg indicated the protective role of ATC in reducing the lipid

peroxidation markers in blood and tissues. Significant increase in the level of endogenous antioxidants such as SOD, CAT, GSH, GPx and GST in cardiac and hepatic tissue indicates the protection against oxidative stress. Cardiac and hepatic tissues showed normal architecture. Genotoxicity analysis by comet assay in tissues treated with MSG and ATC did not show any DNA fragmentation. Administration of ATC protects the hepatic and cardiac function from MSG intoxication as indicated by the significant restoration of serum calcium and functional parameters.

Present study also evaluated the effect of ATC on cell viability, intracellular calcium, lipid peroxidation, antioxidant system and cell damage induced by MSG in rat cardiac myoblast (H9c2) and normal human liver cell (Chang liver). Exposure of H9c2 cells and Chang liver cells to MSG resulted in a concentration dependant decline of cell survival. MSG at 25mM concentration caused significant increase in intracellular concentration of calcium, lipid peroxidation marker with significant decrease of antioxidants. Phase contrast micrograph of H9c2 cells with MSG showed changes in their general cellular morphology as evidenced by swollen cells, irregular membrane shape and cell lysis. Treatment of Chang liver cells with MSG caused marked cell death; existing cells represented condensed nucleus and cytoplasm. Thus the oxidative damage in these cells may be due to the generation of free radicals by excessive influx of calcium. ATC protected the cells against MSG toxicity in a dose dependent manner and optimum protection was observed at 0.4mM concentration in H9c2 cells and Chang liver cells. The exposure of ATC with MSG was found to be significantly effective and showed diminished intracellular calcium, lipid peroxidation and increased antioxidant levels compared with MSG group. Cell lysis was reduced markedly in chang liver cells and completely prevented the morphological changes in H9c2 cells.

To end up with conclusion, present study revealed that the chronic oral exposure of high doses of MSG is potent enough to increase lipid peroxidation and oxidative damages to heart and liver which leads to cardiac and hepatic dysfunction. Supplementation of ATC along with MSG ameliorated lipid peroxidation, increased antioxidant activities and prevents oxidative damages. The mechanisms contributing to its effectiveness involve the ability of ATC to scavenge free radicals and control intracellular calcium. This study suggests that chronic intake of high doses

of MSG has a deleterious effect on hepatic and cardiac cells. Thus people should be aware of the adverse effects of MSG and control the intake of MSG added food. Present study also revealed that even at a higher level of MSG exposure α-tocopherol can exert a protective effect. Thus MSG sensitive people can include α-tocopherol contained oil or α-tocopherol rich food along with MSG suspected food.

References

Adrienne S. (1999). The toxicity of MSG, a study in suppression of information. *Account Res*, 6(4): 259-310.

Beyreuther K, Biesalski HK, Fernstrom JD, Grimm P, Hammes WP, Heinemann U, Kempski O, Stehle P, Steinhart H, Walker R. (2007). Consensus meeting: monosodium glutamate-an update. *Eur J Clin Nutr*, 61:304-313.

Chaudiere J, Ferrar-Iiou R. (1999). Intracellular antioxidants: from chemical to biochemical mechanisms. *Food Chem Toxicol*, 37: 949-962.

FASEB. (1995). Analysis of Adverse Reactions to Monosodium Glutamate (MSG), Report, Life Sciences Research Office, Federation of American Societies for Experimental Biology, Washington, DC.

FAO/WHO. (1974). Toxicological evaluation of certain food additives with a review of general principles and of specifications. 17th Report of the Joint FAO/WHO Expert Committee on Food Additives. FAO Nutrition Meetings Report Series no.53, WHO Technical Report Series no. 539.

Fuke S, Shimizu T. (1993). Sensory and preference aspects of umami. *Trends Food Sci Technol*, 4:246-251.

Geha RS, Beiser A, Ren C, Patterson R, Greenberger PA, Grammer LC, Ditto AM, Harris KE, Shaughnessy MA, Yarnold PR, Corren J, Saxon A. (2000). Multicenter, double-blind, placebo-controlled, multiple-challenge evaluation of reported reactions to monosodium glutamate. *J Allergy Clin Immunol*, 106:973-980.

Gill S, Veinot J, Kavanagh M and Pulido O. (2007). Human heart glutamate receptors-Implications for toxicology, food safety, and drug discovery. *Toxicol Pathol*, 35:411-417.

Havsteen BH. (2002). The biochemistry and medical significance of the flavonoids. *Pharmacol Ther*, 96:67-202.

Inuwa HM, Aina VO, Gabi B, Ola IA, Jaafaru L. (2011). Determination of nephrotoxicity and hepatoxicity of monosodium glutamate (MSG) consumption. *Br J of Pharm and Tox*, 2(3):148-153.

Isa IM, Ghani SA. (2009). A non-plasticized chitosan based solid state electrode for flow injection analysis of glutamate in food samples. *Food Chem*, 112:756-759.

Julio-Pieper M, Flor PJ, DinanTG, John F Cryan. (2011). Exciting times beyond the brain: Metabotropic glutamate receptors in peripheral and non-neural tissues. *Pharmacol Rev*, 63:35-58.

Kwok RHM. (1968). Chinese-restaurant syndrome. *New Engl J Med*, 278:796.

McDermott JH. (2000). Antioxidant nutrients: current dietary recommendations and research update. *J Am Pharm Assoc (Wash)*, 40:785-799.

Monnerie H, Shashidhara S, Le Roux PD. (2003). Effect of excess extracellular glutamate on dendrite growth from cerebral cortical neurons at 3 days in vitro: involvement of NMDA receptors. *J Neurosci Res*, 74:688-700.

Nelson G, Chandrashekar J, Hoon MA, Feng L, Zhao G, Ryba NJ, Zuker CS. (2002). An amino-acid taste receptor. *Nature*, 416:199-202.

Packer L, Landvik S. (1990). Vitamin E in biological systems. In: Emerit, I., Packer, L., Auclair, C. (Eds). Antioxidants in therapy and preventive medicine. Plenum Press, New York, pp. 93-104.

Radnitz C. (1990). Food-triggered migraine, A critical review. *Ann Behav Med*, 12:51-65.

Raiten J D, Talbot JM, Fisher KD. (1995). Executive summary from the report: Analysis of adverse reactions to monosodium glutamate (MSG). *J Nutr*, 125:2892S-2906S.

Woods RK, Weiner J, Abramson M, Thien F, Walters EH. (1996). Patients perceptions of food induced asthma. *Aust N Z J Med*, 26(4):504-512.

Yamaguchi S, Ninomiya K. (1998). What is umami? *Food Rev Int*, 14(3):123-138.

Yatin SM, Varadarajan S, Butterfield DA. (2000). Vitamin E prevents Alzheimer's amyloid beta-peptide (1-42)-induced neuronal protein oxidation and reactive oxygen species production. *J Alzheimers Dis*, 2:123-131.

Published Articles:

1. **Sauganth Paul**, Abhilash Mohanan, Mathews V Varghese, Manju Alex, Harikumaran Nair. (2012). Ameliorative effect of α-tocopherol on monosodium glutamate induced cardiac histological alterations and oxidative stress. ***Journal of the Science of Food and Agriculture,*** 92(15): 3002-3006. (IF: 1.879).
2. **M.V. Sauganth Paul**, M. Abhilash, Mathews V. Varghese, Manju Alex, R. Harikumaran Nair. (2012). Protective effects of α-tocopherol against oxidative stress related to nephrotoxicity by monosodium glutamate in rats. ***Toxicology Mechanisms and Methods,*** 22(8): 625-630. (IF: 1.548).

Published Abstracts:

3. **Sauganth Paul M.V**, Harikumaran Nair R. Monosodium glutamate (food flavour enhancer) impairs cardiac antioxidant system and cytoarchitecture. **25th Kerala Science Congress**, 29th Jan-1st Feb 2013, Technopark, Trivandrum, Kerala, India. pp. 170-172.
4. **Sauganth Paul M.V**, Harikumaran Nair R. Excitotoxic food additive monosodium glutamate - A menace to myocardium. **International Conference On Advances In Biological Sciences**, 15-17 March 2012, Kannur, Kerala, India. pp. 177, MB053.
5. **Sauganth Paul M.V**, Harikumaran Nair R. Long term exposure to monosodium glutamate on antioxidant defense system and histopathology in rat kidney. **International conference on Recent Trends in Therapeutic Advancements of Free Radical Science**, 9-11 January 2011, Chennai, India. pp. 168.
6. **Sauganth Paul M.V**, Harikumaran Nair R. Effect of monosodium glutamate on antioxidants and histopathology in brain. **98th Indian Science Congress**, 3-7 January 2011, SRM University, Chennai, India. pp. 206.
7. **Sauganth Paul M.V**, Harikumaran Nair R. Lipid peroxidation and histopathological alterations in cardiac tissue of monosodium glutamate administered rats. **INDO-US Workshop on Translating Molecular Cardiology into Clinical Practice**, 6-8 August 2010, Rajiv Gandhi Center for Biotechnology (RGCB), Thiruvananthapuram, Kerala, India. pp. 93.